Purification of a Soluble Template-Dependent Rhinovirus RNA Polymerase and Its Dependence on a Host Cell Protein for Viral RNA Synthesis

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The soluble phase of the cytoplasm of human rhinovirus type 2-infected cells contains an enzymatic activity able to copy rhinovirus RNA without an added primer. This RNA-dependent RNA polymerase (replicase) makes a specific copy of the added rhinovirus RNA, as shown by hybridization of the product to its template RNA but not to other RNAs. The same replicase preparation also contains a virus-specific polyuridylic acid [poly(U)] polymerase activity which is dependent on added polyadenylic acid-oligouridylic acid template-primer. Both activities purify together until a step at which poly(U) polymerase but no replicase activity is recovered. Addition of a purified HeLa cell protein (host factor) to this poly(U) polymerase completely reconstitutes rhinovirus replicase activity. Host factor activity can be supplied by adding oligouridylic acid, suggesting that the host cell protein acts at the initiation step of rhinovirus RNA replication. A virus-specific 64,000-dalton protein purifies with both poly(U) polymerase and replicase activities.

MATERIALS AND METHODS

Cells and viruses. HeLa cells were grown in suspension culture (minimal essential medium containing 10% newborn calf serum). HRV-2 strain HGP was obtained from the American Type Culture Collection, Rockville, Md. Initially, HRV-2 was propagated in HeLa cell monolayer cultures six to seven times. Partially purified viruses obtained from monolayer cultures were then adapted to grow in suspension cultures with Joklik minimal essential medium containing 10% newborn calf serum. After the fifth passage through suspension culture of HeLa cells, a virus titer of ca. 10^{10} PFU/ml was obtained. This preparation was used as the virus stock.

Virus infection. Suspension cultures of HeLa cells were infected with HRV-2 at a multiplicity of infection of 80 to 100 PFU per cell. Virus was adsorbed for 30 min at room temperature. Minimal essential medium containing 10% newborn calf serum was then added to the cell suspension, and infection was carried out at 34.5°C. Actinomycin D was added to a concentration of 5 μg/ml in experiments in which it was desirable to inhibit host RNA synthesis. For enzyme preparation, infections were stopped at 7 h and 1,000 ml of cells (4 × 10^7 cells per ml) was collected by centrifugation, washed once in Earle saline, and suspended in 10 ml of 10 mM Tris-hydrochloride (pH 7.5)–10 mM NaCl–1.5 mM MgCl_{2}. A cytoplasmic extract was prepared by breaking the cells in a Dounce homogenizer. The extract was centrifuged at 20,000 × g for 30 min; the supernatant was considered fraction I.

Purification of replicase: phosphocellulose chromatography. Phosphocellulose was prepared by the method of Burgess (8). A column (1 by 20 cm) was equilibrated with buffer A (50
mM Tris-hydrochloride [pH 8.0], 20% [vol/vol] glycerol, 0.5% Nonidet P-40, 5 mM 2-mercaptoethanol). Fraction I was diluted 1:1 with buffer A and loaded on the column at a flow rate of 18 ml/h. The column was then thoroughly washed with buffer A, and protein was eluted with a linear gradient of 0 to 1 M KCl in buffer A. Fractions were assayed for polyadenylic acid [poly(A)]-oligo(U)-directed polyuridylic acid [poly(U)] polymerase activity as well as rhinovirus RNA-dependent RNA polymerase activity. Fractions were pooled, concentrated against solid sucrose to one-third their original volume, and dialyzed overnight against buffer A (fraction II). Replicate prepared this way was stable when stored at -70°C for 2 months, with 10 to 20% loss of activity.

**Poly(U)-Sepharose chromatography.** A poly(U)-Sepharose 4B column (0.8 by 10 cm) was washed thoroughly with 2 M KCl before use. The column was then equilibrated with buffer B (buffer A containing 50 mM KCl). Fraction II enzyme was diluted 1:1 with buffer B and applied to the column at a flow rate of 6 ml/h. The column was thoroughly washed with buffer B. Proteins bound to the column were eluted either batchwise with buffer A containing 0.25 M KCl or with a linear gradient of 50 to 400 mM KCl in buffer A. Fractions were assayed for poly(U) polymerase activity and rhinovirus RNA-dependent replicase activity. Active fractions were pooled, concentrated, and dialyzed against buffer A to yield fraction III (for batch-eluted) or fraction IV (for gradient-eluted) enzyme. Polymerase prepared this way lost 10 to 20% activity when stored at -70°C for 6 weeks.

**Purification of host factor.** Purification of host factor from uninfected HeLa cells to apparent homogeneity was as previously described (11). Purified host factor showed a single 67,000-dalton band on sodium dodecyl sulfate (SDS)-gel electrophoresis followed by Coomassie blue staining.

**Rhinovirus RNA.** Infected cells were collected at 10 to 11 h postinfection, washed once in Earle saline, and suspended in cold 1% Nonidet P-40-10 mM Tris-hydrochloride (pH 8.0)-10 mM NaCl. The nuclei were removed by centrifugation at 5,000 × g for 5 min, and the virions were collected by centrifugation at 45,000 rpm for 2 h in a Beckman type 65 rotor. Virions were suspended in 10 mM Tris-hydrochloride (pH 7.5)-0.1 M NaCl-0.001 M EDTA-0.1% SDS and were purified by sucrose density gradient centrifugation (31). Gradients were fractionated, and optical density at 280 nm was determined for each fraction. Virus peaks sedimenting at ca. 150S were pooled and sedimented again with a Beckman type 65 rotor. The virus pellet was suspended in 10 mM Tris-hydrochloride (pH 7.5)-1 mM EDTA. Viral RNA was prepared by phenol extraction (three times). The RNA was precipitated in 70% ethanol and stored at -70°C in small portions. The integrity of rhinovirus RNA was checked by agarose gel electrophoresis under denaturing conditions (1). The RNA migrated as a single band at ca. 34S. All buffer solutions used in RNA purification procedure were treated with 0.1% diethylpyrocarbonate and autoclaved.

**Enzyme assays.** The poly(A)·oligo(U)-dependent poly(U) polymerase activity was assayed for 30 min at 30°C as described by Flanagan and Baltimore (17). For rhinovirus RNA-dependent replicase activity, the standard incubation mixture contained, in a total volume of 50 μl, 50 mM HEPES (N-2-hydroxyethylpiperazine-N'2-ethanesulfonic acid)-KOH (pH 7.6), 3 mM magnesium acetate, 50 μM ZnCl2, 4 mM dithiothreitol, 10 μg of actinomycin D per ml, 0.2 mM each of three other ribonucleoside triphosphates, 1 to 6 μM [α-32P]UTP (specific activity, 20,000 to 50,000 cpm/pmol), and 0.5 to 1.0 μg of fraction III replicase as defined above.

![Figure 1](http://jvi.asm.org/)

**FIG. 1.** Effect of Mg2+ concentration on rhinovirus replicase activity. Ten micrograms of fraction II replicase was assayed at various concentrations of magnesium acetate in the presence of (○) or absence (□) of added rhinovirus RNA.

Host factor-dependent replicase activity was assayed essentially the same way as the partially purified replicase activity, except that 1 to 2 μg of fraction IV replicase and 0.1 μg of fraction VII host factor (11) were used. RNA synthesis in the presence of only fraction IV replicase served as the control. Incubation was for 1 h at 30°C. The labeled products were collected on membrane filters (0.45 μm) after precipitation with 7% trichloroacetic acid in the presence of 100 μg of added carrier RNA. The filters were dissolved in 5 ml of Bray scintillation fluid (New England Nuclear Corp.) and counted.

**Materials.** All chemicals, unless otherwise stated, were purchased from Sigma Chemical Co., St. Louis, Mo. Unlabeled nucleotides were obtained from Calbiochem-Behring, La Jolla, Calif. Poly(A) was purchased from Miles Laboratories, Inc., Elkhart, Ind. Oligo(U)10-20 was purchased from Collaborative Research, Inc., Waltham, Mass. Poly(U)-Sepharose 4B was obtained from Pharmacia Fine Chemicals, Inc., Piscataway, N.J. Phosphocellulose was purchased from Whatman, Inc., Clifton, N.J. All radioisotopes were purchased from New England Nuclear Corp., Boston, Mass.

**RESULTS**

**Isolation of soluble, template-dependent rhinovirus replicase.** To examine whether a virus-specific RNA-dependent RNA polymerase (replicase) activity could be purified from the soluble phase of cells infected with HRV-2 which can copy exogenously added rhinovirus RNA, we purified it by phosphocellulose chromatography. The soluble form of the enzyme was chosen because it is more readily purified and quite stable. Moreover, previous studies with poliovirus RNA polymerase have shown that it can be readily purified from the cytoplasm of infected cells (13, 19). Figure 1 shows the ability of the phosphocellulose-purified enzyme to copy rhinovirus RNA (plus strand) as a function of Mg2+ concentration. In the presence of Mg2+, the enzyme was able to
incorporate UMP in response to the addition of rhinovirus RNA. Maximum incorporation of UMP was observed at a Mg\(^{2+}\) concentration of 1 to 4 mM. No incorporation was apparent in the absence of added rhinovirus RNA, implying that RNA-dependent RNA synthesis was occurring. All four ribonucleoside triphosphates were needed for optimal activity (data not shown), indicating heteropolymeric RNA synthesis. The Mg\(^{2+}\) optimum for rhinovirus RNA-dependent RNA synthesis was quite broad (Fig. 1).

To determine whether the product of RNA synthesis was a copy of the added viral RNA, we isolated, denatured, and renatured the RNase-resistant product from an in vitro reaction, either by itself or in the presence of either HRV-2 RNA or heterologous RNAs (Table 1). HRV-2 RNA could drive all of the product into a RNase-resistant form, whereas the self-annealed product or the product annealed with heterologous viral or cellular RNAs showed only ca. 25% RNase resistance. This background could be due to partial renaturation of the labeled product with the large molar excess of unlabeled rhinovirus template RNA present in the reaction.

**Replicase activity is rhinovirus specific.** To determine whether the replicase activity was specific to infected cells, we prepared cytoplasmic extracts from infected and mock-infected cells at various times after infection or mock infection. Enzyme was then prepared by eluting infected or mock-infected extracts through phosphocellulose for each time point. No replicase activity was detected in the eluates of mock-infected extracts (Fig. 2). The rhinovirus-specific RNA-dependent replicase activity was first detected at ca. 4 h after infection and increased almost linearly until 7 h after infection. The first increase in enzyme activity coincided with the virus-specific RNA synthesis as measured by actinomycin D-resistant incorporation of \(^{3}H\)uridine in infected cells (Fig. 2). Similar results were reported for the endogenous rhinovirus replicase activity catalyzed by replicase-template complex (34). A poly(A)·oligo(U)-dependent poly(U) polymerase activity also coincided with the replicase and virus-specific RNA synthesis activities (Fig. 2). This activity was not present in uninfected cells and was not induced by mock infection of cells (data not shown). The poly(A)·oligo(U)-dependent poly(U) polymerase activity was first detected in poliovirus-infected cells (17), and this activity is now believed to be associated with poliovirus replicase protein P63 (NCVP-4) (6, 32).

**Further purification and characterization of replicase.** The phosphocellulose-purified (fraction II, Table 2) rhinovirus replicase could be further purified by binding to poly(U)-agarose in low salt and eluting with 0.25 M KCl (fraction III, Table 2). This yielded a fraction ca. 30-fold purified over the crude extracts (Table 2). Both rhinovirus RNA-dependent replicase and poly(A)·oligo(U)-dependent poly(U) polymerase activities co-eluted from the column (data not shown). Attempts to elute the enzyme with a linear salt gradient, however, resulted in complete loss of replicase activity, whereas poly(U) polymerase activity was still evident (Fig. 2).

![FIG. 2. Time course of appearance of rhinovirus replicase.](http://jvi.asm.org/)

### TABLE 1. Hybridization of replicase product to different RNAs\(^a\)

<table>
<thead>
<tr>
<th>Added RNA</th>
<th>% Ribonuclease resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>23</td>
</tr>
<tr>
<td>Rhinovirus type 2</td>
<td>100</td>
</tr>
<tr>
<td>Rhinovirus type 1</td>
<td>26</td>
</tr>
<tr>
<td>Globin messenger</td>
<td>24</td>
</tr>
<tr>
<td>Brome mosaic virus</td>
<td>23</td>
</tr>
<tr>
<td>Total HeLa mRNA</td>
<td>20</td>
</tr>
</tbody>
</table>

\(^a\) \(^{3}P\)ICMP-labeled product of fraction II (phosphocellulose purified) rhinovirus replicase copying rhinovirus type 2 RNA was prepared. The double-stranded RNA product was isolated by phenol extraction after treatment in vitro-synthesized RNA with pancreatic RNase as described previously (13). About 3 ng (20,000 cpm) of double-stranded RNA product was dissolved in 20 μl of water and boiled for 5 min in the presence of 50 ng of the indicated added RNA. The mixture was adjusted to 0.5% SDS-0.45 M NaCl-0.045 M sodium citrate and allowed to hybridize at 70°C for 1 h. The sample was chilled and divided into equal portions. One portion was treated with 100 μg of pancreatic RNase per ml and 20 μl of T1 RNase per ml. Acid-precipitable radioactivity was determined. Background radioactivity from a boiled but unannealed sample was subtracted (ca. 5%), and the percent RNase resistance was calculated.

### TABLE 2. Purification of rhinovirus-specific poly(U) polymerase\(^a\)

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total protein (mg)</th>
<th>Total activity (U)(^b)</th>
<th>Sp act (U/mg)</th>
<th>Purification (fold)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. S(_{50})</td>
<td>500</td>
<td>300,000</td>
<td>600</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>II. Phosphocellulose</td>
<td>40</td>
<td>200,000</td>
<td>5,000</td>
<td>8</td>
<td>75</td>
</tr>
<tr>
<td>III. Poly(U)-Sepharose</td>
<td>5</td>
<td>100,000</td>
<td>20,000</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>IV. Poly(U)-Sepharose</td>
<td>1</td>
<td>59,000</td>
<td>59,000</td>
<td>98</td>
<td>20</td>
</tr>
</tbody>
</table>

\(^a\) Rhinovirus type 2-infected HeLa cells were collected 7 h after infection, and viral poly(U) polymerase was purified by the method described in the text.

\(^b\) One unit of poly(U) polymerase activity is defined as the amount of protein required to catalyze the incorporation of 1 pmol of labeled UMP into acid-precipitable form at 30°C for 30 min under the poly(U) polymerase assay conditions (17).
3). Purification of poly(U) polymerase by means of gradient elution from poly(U)-agarose gave an overall 100-fold enrichment of poly(U) polymerase activity (Table 2). We have designated the final fraction as fraction IV poly(U) polymerase. When individual fractions containing [35S]methionine-labeled viral proteins from the poly(U)-agarose column (gradient elution) were analyzed by SDS-polyacrylamide gel electrophoresis, only one viral protein having an approximate molecular weight of 64,000 was found to correspond exactly to poly(U) polymerase activity (Fig. 3).

The best studied picornaviral replicase is that of poliovirus (4, 11, 13, 15, 17–19, 32, 33). In its purified form, the poliovirus RNA polymerase requires a host cell protein (host factor) to copy viral RNA in vitro (15). To determine whether rhinovirus replicase activity is also dependent on host factor, we pooled and concentrated fractions (numbers 8 to 12, Fig. 3) containing poly(U) polymerase activity and tested the concentrated enzyme for its ability to copy viral RNA in the absence and presence of host factor. The concentrated enzyme was still inactive in copying virion RNA (Fig. 4A). However, when purified host factor was added to this fraction, it completely reconstituted replicase activity (Fig. 4A). RNA synthesis in response to rhinovirus RNA increased linearly with increasing concentrations of poly(U) polymerase in the presence of a saturating amount of host factor. When the viral polymerase was omitted from the reaction, there was virtually no RNA synthesis, indicating that both viral and host components were necessary for RNA synthesis. Oligo(U), a synthetic primer, was able to substitute for host factor (Fig. 4C). RNA synthesis in the presence of a constant amount of viral polymerase increased linearly with increasing concentrations of host factor or oligo(U) (Fig. 4B and C). Oligo(U) was maximally active at a ratio of 5 mol of oligo(U) to 1 mol of rhinovirus RNA and was inhibitory at higher concentrations. Poliovirus-specific RNA-dependent RNA polymerase also utilizes oligo(U) for copying of poliovirus RNA. However, with poliovirus replicase, maximal stimulation is observed at an oligo(U) to poliovirus RNA ratio of 20:1 (15).

Stimulation of replicase activity by host factor is dependent upon the initial concentration of host factor. At low concentrations of host factor, the rate of synthesis is proportional to the host factor concentration. A typical time course for the host factor-dependent replicase reaction is shown in Fig. 5A. The reaction was linear for almost 3 h, after which RNA synthesis reached a plateau.

The pH optimum for rhinovirus replicase reaction was quite broad (Fig. 5B). Maximal RNA synthesis was observed at pH 7.6. Eighty to 90% of total RNA was synthesized at pH values as low as 7.4 and as high as 8.0 compared with the value at pH 7.6. The pH optimum for poliovirus replicase was previously found to be quite sharp at pH 8.0 (unpublished data).

Like poliovirus replicase, rhinovirus RNA replicase was quite sensitive to salt. Over 90% RNA synthesis was inhibited at 50 mM KCl (Fig. 6A). For poliovirus replicase, however, 100 mM KCl was required to obtain an 80 to 90% inhibition of RNA synthesis (13). Similar results were obtained for both rhinovirus and poliovirus replicases with NaCl (data not shown).

In contrast to monovalent salts, Zn2+ (in the form of ZnCl2) was found to stimulate host factor-dependent rhinovirus replicase activity (Fig. 6B). This stimulation was concentration dependent. A maximal stimulation of fourfold, compared with the control without Zn2+, was observed at 50 μM Zn2+. However, Zn2+ was not able to substitute for Mg2+ (data not shown). The effect of Zn2+ was most probably at the level of RNA chain elongation, since oligo(U)-primed copying of both pure poly(A) and rhinovirus RNA was stimulated by zinc (data not shown).

**Template specificity of rhinovirus replicase.** To examine the template specificity of the replicase, we tested the most purified (fraction IV, Table 2) enzyme preparation for its ability to copy a number of RNAs. In the presence of purified host factor, the fraction IV replicase did not show RNA specificity in copying rhinovirus RNA over copying host genome poly(A)-containing RNA templates (Table 3). Although rhinovirus RNA was copied with the highest efficiency, the replicase also copied poliovirus RNA and globin mRNA with an efficiency of 50 to 60% compared with that with

![FIG. 3. Analysis of viral proteins present in fractions from a poly(U)-Sepharose 4B column having rhinovirus-specific poly(U) polymerase activity. (A) Rhinovirus proteins were labeled with [35S]methionine (1,070 Ci/mmol, 2 nCi per 4 × 10⁶ cells; New England Nuclear Corp.) by the procedure described by Yin and Lomax (35). Purification of poly(U) polymerase through phosphocellulose, poly(U)-Sepharose 4B (step elution), and poly(U)-Sepharose 4B (gradient elution) was carried out as described in the text. Fractions from the final poly(U)-Sepharose 4B (fraction IV, Table 2) column were assayed for poly(U) polymerase activity (●) as described by Flanagan and Baltimore (17) and for rhinovirus RNA-dependent replicase activity (○). Labelled products were collected on membrane filters and counted. (B) Corresponding fractions containing [35S]methionine-labeled viral proteins were analyzed on a 12.5% SDS-polyacrylamide gel (28) alongside [35S]methionine-labeled rhinovirus-infected extract (extreme right lane). The gel was fixed with 10% acetic acid and fluorographed, and then an autoradiogram was prepared. Migrations of standard protein markers on the same gel are indicated.](http://jvi.asm.org/)
rhinovirus RNA. With brome mosaic virus RNA as a template lacking the 3′-terminal poly(A) tail, however, the copying was significantly less (Table 3).

**DISCUSSION**

It is evident that the soluble fraction of rhinovirus-infected cells contains a virus-specific RNA replicase activity that can be purified to demonstrate copying of exogenously added rhinovirus RNA in vitro. Detection and purification of this RNA replicase was greatly facilitated by an assay for poly(U) polymerase activity in response to poly(A)·oligo(U). Even the purest replicase preparation contains this activity (Fig. 3), implying that the replicase and poly(U) polymerase are probably closely related activities.

The soluble replicase activity contains mainly one viral protein with an approximate molecular weight of 64,000 (P64) (Fig. 3). However, in some preparations we detected two other viral proteins of approximate molecular weights 74,000 and 45,000. These two proteins can also be detected in the [35S]methionine-labeled viral replicase preparation shown in Fig. 3, but only after pooling and concentrating peak fractions and overexposing the autoradiogram. The rhinovirus-specific P64 may be analogous to poliovirus RNA polymerase (P63) that has already been shown to contain both replicase and poly(U) polymerase activities (6, 32).

It is clear that when the rhinovirus replicase preparation described here is further purified, the enzyme becomes dependent on an added protein factor found in uninfected

**FIG. 4.** Requirement for rhinovirus RNA-dependent replicase activity. (A) Reactions in a 50-μl volume were carried out by using 1 μg of rhinovirion RNA for 1 h at various concentrations of fraction IV poly(U) polymerase in the absence (○) or presence (●) of 0.36 μg of fraction VII host factor (11). (B and C) Similar reactions were carried out with various concentrations of either fraction VII host factor (B) or oligo(U) (C) in the absence (○) or presence (●) of 1 μg of fraction IV poly(U) polymerase.

**FIG. 5.** Kinetics and pH optimum of rhinovirion RNA-dependent RNA synthesis catalyzed by viral poly(U) polymerase and host factor. (A) One microgram of fraction IV poly(U) polymerase was incubated with 0.3 μg of fraction VII host factor (11) under RNA synthesis assay conditions. The reactions were stopped at the indicated times and processed. (B) Rhinovirion RNA-dependent RNA synthesis was carried out as described in A, except that the pH of the buffer solution was varied as indicated. Both Tris-hydrochloride and HEPES-KOH buffers gave similar results.

**FIG. 6.** Effect of KCl and Zn2+ on rhinovirus replicase activity. (A) Rhinovirion RNA-dependent RNA synthesis was carried out as described in the legend to Fig. 5, except that the indicated amounts of KCl were added at the start of reactions. (B) Rhinovirion RNA replicase activity was determined in the absence or presence of the indicated amounts of ZnCl2.
TABLE 3. Template specificity of rhinovirus replica*

<table>
<thead>
<tr>
<th>Fraction IV poly(U) polymerase</th>
<th>Host factor</th>
<th>RNA</th>
<th>[3P]UMP incorporation (pmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>None</td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>+</td>
<td>Rhinovirus</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>Poliovirus</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>Globin</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>BMV*</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>Polio/Rhinovirus/Globin/BMV</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>Polio/Rhinovirus/Globin/BMV</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

* Reaction mixtures contained 1 μg of fraction IV rhinovirus RNA polymerase, 1 μg of various RNAs as indicated, and 0.1 μg of purified host factor. Assay conditions were the same as described in the text.

* BMV, Brome mosaic virus.

cells (host factor [14, 15]). This is not surprising because we previously showed that the same host cell protein was required for replication of poliovirion RNA in vitro (4, 12, 15). Host factor is a single polypeptide of apparent molecular weight 67,000 (3, 11). Most of this protein is soluble; however, 30% of total host factor is found associated with ribosomes (12). The host factor estimated to be present at 50,000 to 100,000 copies per cell (3, 12) interacts physically with poliovirus replica (3, 12). Our unpublished results indicate that this protein is present in phosphocellulose-purified rhinovirus replica preparation. During salt gradient elution from poly(U)-Sepharose 4B, the host factor activity elutes before virus-specific polymerase activity (data not shown). The rhinovirus replica activity can be reconstituted by combining this host factor activity with viral polymerase from the same column. This result strongly suggests that replica itself has not simply been altered during purification and that host factor activity was indeed present in the starting material. Consistent with this notion is the result that nonspecific proteins like bovine serum albumin or ovalbumin do not stimulate rhinovirus replica activity (data not shown).

At its present stage of purity, the rhinovirus replica does not show any significant preference for rhinovirus RNA as a template. It copies other RNAs, both viral and cellular. This is puzzling, because the viral replica apparently specifically copies viral RNA in the cytoplasm of infected cells. The reason for this is not clear, but it could be due to contaminating host proteins in the preparation. Alternatively, the replica used in these experiments may be deficient in a protein required for specific copying of the rhinovirus RNA template. The rhinovirus replica copies a synthetic homopolymer, poly(A), in the presence of a synthetic primer, oligo(U). At present, we do not know whether other homopolymeric RNAs like poly(G), poly(C), and poly(U) will be copied by the replica in the presence of appropriate primers.

How the replica initiates copying of viral RNA template remains obscure. For poliovirus RNA replication, results from our laboratory as well as others suggest that a precursor(s) to poliovirus genome-linked protein may act as a primer for initiation of poliovirus minus-strand RNA synthesis (5, 28, 29). It is not known at present whether there is a similar protein attached to the 5' terminus of rhinovirus RNA. Clearly, more studies are required to answer this question.

Our preliminary experiments in characterizing the products of in vitro replica reaction have revealed the production of labeled cRNA molecules as big as 28S. So far, we have not been able to synthesize complete, full-length copies of the template RNA. At present we do not know the reason for this. One possibility is the limited degradation of the input viral RNA in the in vitro reaction. However, the replica and host factor preparations used in these studies do not contain detectable amounts of RNase (data not shown).

To date, the best studied picornaviral replica is that of poliovirus. There are some very interesting similarities between poliovirus and rhinovirus replicas. First, in their purified forms, both polymerases are dependent on the same host cell protein (host factor). For both of these enzymes, the requirement for host factor can be met by a synthetic primer, oligo(U). With poliovirus RNA polymerase, oligo(U) was shown to prime RNA synthesis by binding to the poly(A) tract at the 3' end of viral RNA (4, 33). We assume, although we have not shown, that rhinovirus replica can elongate an oligo(U) primer hydrogen bonded to the 3'-terminal poly(A) tract of rhinovirus RNA. Both polio- and rhinovirus polymerases are stimulated by Zn+2 and inhibited by monovalent salts. It is interesting to note that all nuclear acid polymerases examined to date have been zinc-containing enzymes (27). It is possible that zinc is partially or totally removed during purification of rhinovirus replica and so the purified enzyme shows a requirement of zinc for its activity. In this context, it is important to note that chelating agents that specifically chelate zinc inhibits residual polio- and rhinovirus replica activity in the absence of zinc (4).

It is surprising to find most of the replica in the soluble phase of the cell, because viral RNA replication occurs on cellular membranes (10). A reasonable explanation, as noted previously by Dasgupta et al. (13), is that initiation of RNA synthesis occurs by interaction of soluble replica and viral RNA and thereafter the complex may bind to membranes. This explanation is consistent with the facts that all template-dependent picornavirus replica preparations reported to date have been cytoplasmic (3, 11, 13, 15, 17-19, 32; this report) and the majority of the host factor is localized in the soluble phase of cells (3, 12). Moreover, it is worth mentioning that the in vitro system reported here synthesizes minus strands, whereas previous studies on intracellular viral RNA synthesis have concentrated mainly on plus-strand synthesis. The requirements for plus-strand synthesis and minus-strand synthesis could be significantly different, as noted previously for the replicas of RNA bacteriophages (16, 22, 25).

Finally, the establishment of a template-dependent replication system from HRV-2-infected cells gives us an opportunity to compare the mechanism of initiation of RNA replication by two viruses, rhinovirus and poliovirus, both belonging to the picornaviridae family.

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LITERATURE CITED


